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CHARACTERISTICS OF HISTAMINE RELEASE EVOKED BY ACETYLCHOLINE IN ISOLATED RAT MAST CELLS

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SUMMARY

1. Histamine secretion from rat mast cells occurs in the presence of nanomolar concentrations of acetylcholine.

2. Intact glycolytic and oxidative metabolism is required for the acetylcholine-induced histamine secretion. Removal of extracellular glucose, hypoxia, cyanide and monoiodoacetate almost completely inhibit the secretion.

3. The secretion of histamine is dependent on the extracellular H ion concentration and is blocked when the cells are exposed to Na-deficient media.

4. The order of potency of cholinergic agonists in evoking the secretion of histamine is oxotremorine > acetylcholine > choline > carbamylcholine > nicotine.

5. Atropine competitively blocks the acetylcholine-induced histamine secretion, indicating the presence of cholinergic muscarinic receptors on mast cells.

6. Dibutyryl cyclic AMP and adrenaline inhibit the acetylcholine-induced histamine secretion, indicating a regulatory function afforded by cyclic nucleotides in the cholinergic histamine release.

INTRODUCTION

A link between the cholinergic system and histamine stores has been established since the demonstration that vagal stimulation leads to release of gastric histamine (Kim & Shore, 1963; Shore, 1965; Kahlson, Rosengren & Thurnberg, 1967). In the submaxillary and parotid glands of cats and dogs, cholinergic agonists or the stimulation of cholinergic nerves produce a significant depletion of tissue histamine stores (Werle & Lorenz, 1966; Erjavec, Beaven & Brodie, 1967; Lorenz, Haubensack, Hutzel & Werle, 1968; Lorenz, Huhndt, Kusche, Barth, Haubensack, Hutzel, Schmal, Gerant, Wächter, Matejka, Hahn & Werle, 1973; Erjavec, 1978). The same link has been further enforced by the observations of Kaliner, Orange & Austen (1972*a*), Kaliner, Wasserman & Austen (1973), Kaliner & Austen (1975), showing that acetylcholine or carbamylcholine enhances antigen-induced histamine release from IgE sensitized tissues. Moreover, it has been shown that carbamylcholine increases histamine release induced by 48/80 in isolated mast cells (Sullivan, Parker, Eisen & Parker, 1975*a*).

A possible relationship between cholinergic activity and the secretory response of mast cells *in vivo* has been recently proposed by Cho & Ogle (1979). They have shown

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that atropine injected subcutaneously antagonizes stress-induced decrease in mast cell count in the gastric glandular mucosa of pylorus-occluded rats.

With regard to the action of acetylcholine on histamine-containing cells, isolated purified rat mast cells secrete histamine when exposed to acetylcholine in a dose-dependent fashion, through a phenomenon which is dependent on temperature and the presence of extracellular Ca and glucose. Atropine blocks acetylcholine-induced histamine secretion (Fantozzi, Masini, Blandina, Mannaioni & Bani-Sacchi, 1978*a*; Fantozzi, Moroni, Masini, Blandina & Mannaioni, 1978*b*). Our purpose in the present paper is to enlarge on the characteristics of histamine secretion evoked by acetylcholine.

METHODS

Male Wistar albino rats weighing between 200 and 400 g were used for the experiments. Rats were anaesthetized in an atmosphere of ether and air and then killed by decapitation. Eight ml. saline (NaCl, 154 mM) adjusted to pH 6 with 10% Sörensen phosphate buffer was injected into the peritoneal cavity of each rat, and 2 ml. of the same solution was injected into the pleural cavities. After having massaged the abdomen for 1 min, the fluids were withdrawn from the peritoneal cavity, opened with a mid-line incision, and from the pleural cavity, opened on the diaphragm just below the xiphoid process. Peritoneal and pleural washings contain about 5% mast cells. Separation of mast cells from the other cells was achieved by density gradient centrifugation in Ficoll (molecular weight 370,000, Pharmacia Co., Uppsala, Sweden) dissolved in a solution with the following composition: NaCl, 145 mM; KCl 2·4 mM; $CaCl_2$ 0·9 mM; human serum albumin (Behringwerke) 0·1%, adjusted to pH 7·4 with Sörensen phosphate buffer. The gradient comprises a lower layer of 1 ml. Ficoll (40% by weight) and an upper layer of 1 ml. Ficoll (30% by weight) into which about 8 ml. peritoneal washings and 2 ml. pleural washings were placed. The tubes were centrifuged at 300 *g* for 10 min at 0–4 °C. The mast cells formed a pellet at the base of the tube, the other cells remaining at the interface of the two concentrations of Ficoll. More than 80% of the cells in the pellet were mast cells. The cells were washed free of Ficoll by resuspension in 5 ml. of a medium of the following composition: NaCl 145 mM; KCl 2·4 mM; $CaCl_2$ 0·9 mM; human serum albumin 0·1%; adjusted to pH 7·4 with Sörensen phosphate buffer. The cells were centrifugated twice at 300 *g* for 10 min at 0–4 °C, before being pooled to provide sufficient cells for one experiment. Unless otherwise indicated, the incubation medium in which the experiments were carried out had the same composition as that used to wash the cells.

Experiments were performed to study the effect of sodium deprivation on acetylcholine-induced histamine release. Na-deficient solutions were obtained by replacing all NaCl with an osmotically equivalent amount of a Na substitute: LiCl, KCl, each 145 mM; $MgCl_2$ 106 mM and adjusted to pH 7·4 with KOH (Cochrane & Douglas, 1976). In order to study the pH-dependence of the release process, the solutions were adjusted to the required pH by adding different volumes of the Sörensen phosphate buffer.

Treatment with drugs

Cholinergic agonists

50 µl. cell suspension were added to test tubes containing various concentrations of acetylcholine, oxotremorine, choline, carbamylcholine and nicotine. The drugs had been dissolved in 2 ml. of the incubation medium. The number of cells added to each tube was about 5×10^4. The samples were incubated at 37 °C in a metabolic shaker for 10 min. The gas phase was air, unless otherwise specified. The reaction was stopped by chilling the tubes in an ice-water bath. Cells were then separated from the medium by centrifugation (400 *g* for 5 min) at 4 °C, and histamine was measured in the supernatants and in the pellets.

Atropine

50 μl. cell suspension (number of cells: 5×10^4) were added to test tubes containing various concentrations of atropine. Incubation at 37 °C was allowed to proceed for 15 min. At the end of incubation the cells were harvested by centrifugation, and resuspended in a medium containing acetylcholine. The same procedure was used with control cells, except that they were incubated for 15 min in a medium which did not contain atropine. Both control and atropine-pretreated cells were incubated with acetylcholine for 10 min, before being divided into supernatants and pellets for the histamine assay.

Dibutyryl cyclic AMP and adrenaline

Treatment with dibutyryl cyclic AMP and adrenaline was carried out as follows. 50 μl. cell suspension (number of cells: 5×10^4) were added to test tubes containing the drug under study dissolved in 2 ml. of the incubation medium. The incubation was started, and after 15 min acetylcholine (20 μl. of a solution suitable for giving the 10^{-10} M final concentration) was added directly to test tubes. The same procedure was used for control cells, except that they were incubated for 15 min in a medium not containing drugs. Incubation at 37 °C was allowed to proceed for 10 min after adding acetylcholine; the subsequent steps were as described above.

Assay

Experiments were performed to ascertain whether acetylcholine is quantitatively recovered after incubation for 1 hr in a medium containing mast cells. Concentrations of acetylcholine of the same order as those used throughout the experiments were added to a medium containing mast cells. Initial and final samples of the supernatants were assayed biologically on the leech dorsal muscle (Murnaghan, 1958); no significant loss of acetylcholine was detected, showing that rat mast cells do not inactivate acetylcholine.

Histamine was measured fluorimetrically using the method of Shore, Burkhalter & Cohn (1959) as modified by Kremzner & Wilson (1961). In the supernatants, *O*-phthaldialdehyde was added directly to the sample after alkalinization. The same procedure was used for the cells, after extraction with HCl (0·1 M) using the method of Bergendorff & Uvnäs (1972). In some experiments the histamine assay was carried out in the same sample both through the fluorimetric assay and through the bio-assay in the presence of atropine and methysergide (Giotti, Guidotti Mannaioni & Zilletti, 1966). The two methods gave comparable results. The authenticity of histamine in control and treated cells was demonstrated either by recording the excitation and fluorescence spectra or through bioassay according to a two by two design (Schild, 1942). Histamine release (supernatant histamine) was expressed as a percentage of the total present in the cells plus supernatant. Spontaneous histamine release ranged between 1 and 8% and was subtracted from all values.

Drugs

The chemicals used to prepare the solutions for the fluorimetric assay were of Suprapur quality, E. Merck, AG. Acetylcholine chloride (batch numbers: 6200920 and 1097392), NaCN and *O*-phthaldialdehyde were obtained from B.D.H. chemicals Ltd. Dibutyryl cyclic AMP, choline chloride (batch number 44C-0950) and carbamylcholine chloride (batch number C-4382) were obtained from Sigma Chemical Co. Other drugs used were: histamine dihydrochloride, Calbiochem; atropine sulphate, Merck; nicotine bitartrate (batch number 663960), B.D.H. Chemicals Ltd; oxotremorine sesquifumarate (batch number 110961), Aldrich Chemical Co. Inc.; compound 48/80, Burroughs Wellcome & Co.

RESULTS

Effects of metabolic inhibition

Previous experiments have demonstrated that the secretory response of mast cells to acetylcholine is blocked when the incubation is carried out at 0–4 °C, or when glucose is withdrawn from the medium (Fantozzi, Masini, Blandina, Mannaioni &

Bani-Sacchi, 1978*a*). In the present experiments, hypoxia obtained through a 10 min incubation under nitrogen produced an almost complete inhibition of histamine secretion in the presence of acetylcholine. Addition to the extracellular medium of cyanide blocked the release of histamine evoked by acetylcholine in a dose-dependent fashion. Monoiodoacetate also inhibited the secretory process.

TABLE 1. Effect of metabolic inhibitors on acetylcholine-induced histamine release

	Histamine release (%, mean ± S.E. of mean, $n = 4$)	
	Control	Acetylcholine (10^{-10} M)
	6·0 ± 1·5	80·2 ± 0·3
Hypoxia	1·1 ± 0·4	1·4 ± 0·2
Monoiodoacetate (10 μM)	—	1·3 ± 0·1
Monoiodoacetate (100 μM)	1·0 ± 0·3	1·2 ± 0·4
Cyanide (25 μM)	—	79·7 ± 2·5
Cyanide (50 μM)	5·5 ± 1·2	5·2 ± 1·5

Effects of H ion concentration and of various Na-deficient media

Histamine release by acetylcholine in isolated purified rat mast cells requires calcium (Fantozzi *et al.* 1978*a*; Fantozzi, Moroni, Masini, Blondina & Mannaioni, 1978*b*). Since it is known that H^+ and Ca^{2+} interact in the antigen-evoked secretion of histamine (Mongar & Schild, 1958), it was of interest to see if any change in pH of the extracellular medium could modify the secretion of histamine caused by acetyl-

TABLE 2. Effect of H ion concentration on acetylcholine-induced histamine release

	Histamine release (%, mean ± S.E. of mean, $n = 4$)	
pH	Control	Acetylcholine (10^{-10} M)
6·4	< 1	< 1
6·9	< 1	66·34 ± 0·78
7·4	< 1	75·60 ± 3·71
7·8	< 1	72·58 ± 2·08
8·2	52·35	73·75 ± 2·75

TABLE 3. Histamine release from rat mast cells incubated 10 min in various Na-deficient media in the presence or absence of acetylcholine

	Histamine release (%, mean ± S.E. of mean, $n = 4$)	
	Control	Acetylcholine (10^{-10} M)
NaCl (145 mM)	2·5 ± 0·7	85·2 ± 0·8
NaCl substitute		
KCl (145 mM)	1·2 ± 0·1	2·3 ± 0·4
LiCl (145 mM)	3·8 ± 0·7	4·1 ± 0·4
$MgCl_2$ (106 mM)	1·1 ± 0·2	1·3 ± 0·3

choline. In the pH range tested (6·4–8·2) an optimum histamine secretion occurred at pH 7·4 and changes of pH from 6·9 to 7·8 did not modify the acetylcholine-induced histamine secretion. However when mast cells were suspended in more acidic or alkaline media cholinergic histamine release was blocked.

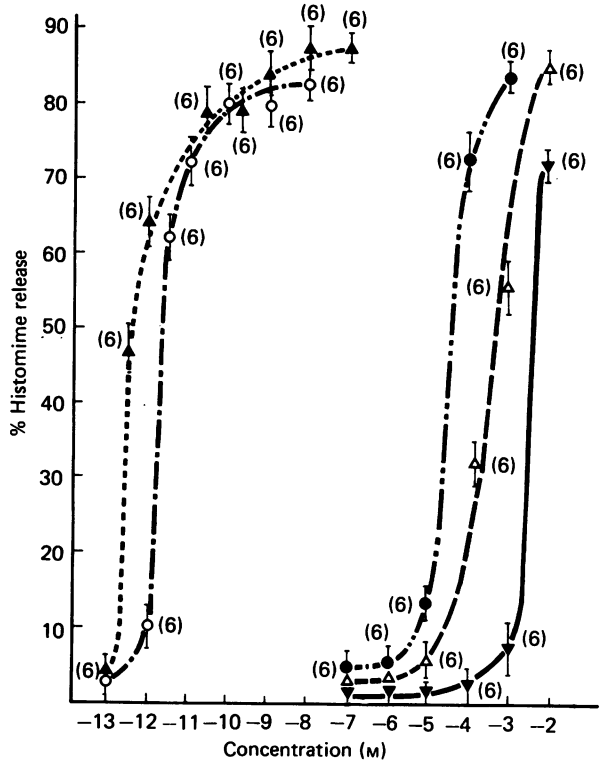

Fig. 1. Log dose–response curves for cholinergic agonists-induced histamine release from rat mast cells. ▲---▲, oxotremorine; ○—○, acetylcholine; ●---●, choline; △—△ carbamylcholine; ▼—▼, nicotine. The figures in parentheses indicate the number of experiments and the vertical bars represent the S.E. of mean.

It has been shown that withdrawal of Na from the extracellular environment may lead to failure of secretion in various cells (Hales & Milner, 1968; Banks, Biggins, Bishop, Christian & Currie, 1969; Parsons, 1970; Martinez & Peterson, 1972; Case & Clausen, 1973). The spontaneous histamine release was not affected when isolated purified rat mast cells were exposed for 10 min to a medium in which all the NaCl was replaced by osmotically equivalent amounts of LiCl, $MgCl_2$, KCl; under these conditions, the release of histamine evoked by acetylcholine was completely prevented.

It is worth noting that preincubation of cells with tetrodotoxin, at concentrations capable of blocking the sodium fast channels (3×10^{-6} M; Kao, 1966), fails to influence the secretion of histamine in the presence of acetylcholine.

Effects of cholinergic agonists

Muscarinic agonists (acetylcholine, carbamylcholine, oxotremorine) were tested for their histamine releasing properties in rat mast cells. The pattern of activity is oxotremorine > acetylcholine > carbamylcholine; choline still retains some histamine releasing properties, while nicotine releases histamine only at very high concentrations, as shown in Fig. 1.

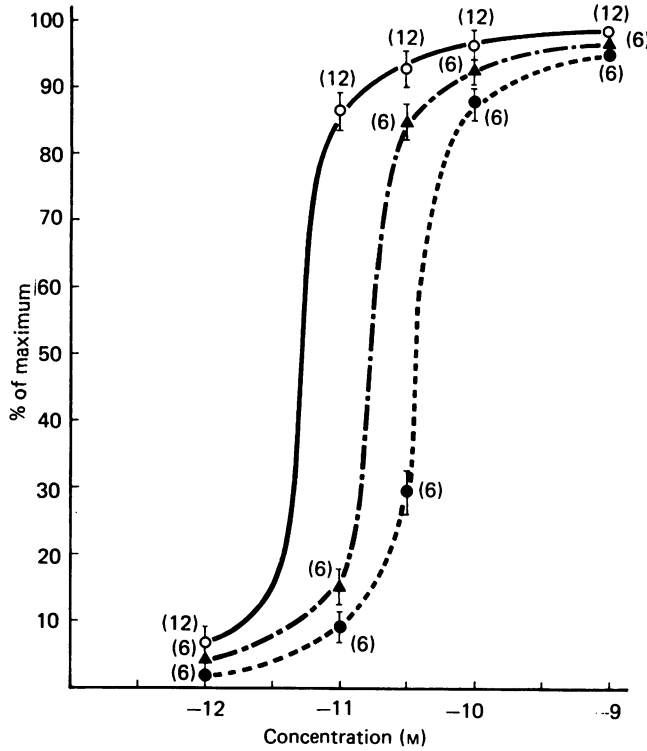

Fig. 2. Effect of atropine on the concentration–response relationship for acetylcholine-induced histamine release from rat mast cells. ○—○, control; △—△, atropine 10^{-8} M; ●---●, atropine 10^{-7} M. The figures in parentheses indicate the number of experiments and the vertical bars represent the S.E. of mean.

Effects of atropine

Atropine blocked acetylcholine-evoked histamine release both when it was added together with acetylcholine and when the cells were preincubated and washed before exposure to acetylcholine (Fantozzi *et al.* 1978*a*). In order to better define the nature of the antagonistic action of atropine, experiments were carried out to study the dose–response curves of acetylcholine in the presence of atropine. The results shown in Fig. 2 indicate that, in the presence of atropine (10^{-8}, 10^{-7} M), the dose–response curves of acetylcholine were shifted to the right in a parallel fashion, always reaching their maximum. The pA_2 value for atropine was calculated according to Van Rossum (1963) and was found to be $8{\cdot}28 \pm 0{\cdot}10$.

Effects of dibutyryl cyclic AMP and of adrenaline

Cyclic nucleotides regulate many secretory processes, including the selective release of histamine (Kazimierczak & Diamant, 1978). Dibutyryl cyclic AMP, 10^{-3} M, did not modify spontaneous histamine release, whereas the percentage of acetylcholine-induced histamine release was significantly reduced by preincubating the cells with this drug.

TABLE 4. Concentration–response relationship for inhibition by dibutyryl cyclic AMP of acetylcholine-induced histamine release. Mean values ± S.E. of six experiments

Concentration of dibutyryl cyclic AMP (M)	Concentration of acetylcholine (M)	Histamine release (%)
—	—	6·9 ± 2·1
—	10^{-10}	72·5 ± 1·7
10^{-3}	—	7·1 ± 3·5
10^{-3}	10^{-10}	4·1 ± 1·6
10^{-4}	10^{-10}	4·3 ± 0·7
10^{-5}	10^{-10}	5·2 ± 1·6
10^{-6}	10^{-10}	76·3 ± 1·8

TABLE 5. Effect of adrenaline on acetylcholine-induced histamine release Mean values ± S.E. of six experiments

Concentration of adrenaline (M)	Concentration of acetylcholine (M)	Histamine release (%)
—	—	3·8 ± 1·2
—	10^{-10}	81·6 ± 2·8
10^{-3}	—	4·1 ± 0·8
10^{-3}	10^{-10}	3·4 ± 0·4
3×10^{-4}	10^{-10}	4·2 ± 1·1
10^{-4}	10^{-10}	82·1 ± 2·5
10^{-5}	10^{-10}	80·7 ± 0·9

Preincubation of the cells with adrenaline at concentrations capable of increasing the intracellular levels of cyclic AMP (Sullivan, Parker, Stenson & Parker, 1975*b*) completely blocked acetylcholine-evoked histamine release.

DISCUSSION

The secretory process evoked by acetylcholine resembles that produced by the well known secretagogues antigen, 48/80 and the ionophoreous antibiotics A 23187 and X537 A. In fact it is exocytotic, as shown by light and electron microscopy (Fantozzi *et al.* 1978*a*, 1978*b*); it is energy requiring, occurs at physiological pH, and needs calcium as well as sodium in the extracellular medium.

One difference however between the secretory processes induced by acetylcholine and by antigen or compound 48/80 lies in the metabolic requirement needed to evoke exocytosis. Acetylcholine causes a secretory response which is both dependent

on glycolytic and oxidative metabolism, while in the case of antigen or compound 48/80 anaerobic glycosysis has been shown capable of producing enough energy for the secretory processes induced by these stimuli even when the oxidative pathway is blocked (Kazimerczak & Diamant, 1978). Therefore anaerobic glycolysis alone cannot furnish sufficient ATP for cholinergic histamine release, unlike that shown for antigen or 48/80-evoked histamine release (Diamant, 1975; Kazimierczak & Diamant, 1978).

Our previous studies (Fantozzi *et al.* 1978*a*; Fantozzi *et al.* 1978*b*) have demonstrated that acetylcholine-induced histamine secretion requires intracellular and extracellular calcium. In the experiments presented in this paper, we have extended the study of ionic dependence of cholinergic histamine release to sodium and hydrogen ions, previously shown to influence the secretory response by various histamine-releasing agents, ATP, compound 48/80 and the ionophore X537 A (Mongar & Schild, 1958; Uvnäs & Thon, 1961; Dahlquist, Diamant & Krüger, 1974; Hayashi, Ichikawa, Saito & Tomita, 1976; Kazimierczak, Patkar & Diamant, 1978). The Present results indicate that monovalent cations are inherent in the mechanism by which acetylcholine releases histamine from rat mast cells. In fact, cholinergic histamine release requires extracellular Na, since no secretion occurs when sodium is substituted isoosmotically with Li, Mg and K. The Na requirement for acetylcholine-evoked histamine secretion agrees with the cationic exchange mechanism suggested by Uvnäs (1964, 1972) for the release of biogenic amines from mast cells. Moreover, the inefficiency of tetrodotoxin in influencing the cholinergic secretory response may exclude the presence of sodium fast channels in the mast cell membrane. Acetylcholine-induced histamine release is also dependent on extracellular H ion concentration, an optimum secretion occurring over the pH range 6·9–7·8, beyond which there is a sharp decrease in histamine release. The dependence on extracellular pH of antigen- or 48/80-stimulated histamine secretion has been previously demonstrated (Mongar & Schild, 1958; Chakravarty, 1960; Uvnäs & Thon, 1961; Foreman, Hallett & Mongar, 1977). Foreman *et al.* (1977) suggested that the influence of H ions on histamine release may be the result of variations in permeability of the mast cell membrane to calcium occurring at different pH. Ca and H ions might interact in a similar way also in cholinergic histamine release.

It may be that the cause of active and ionic-dependent acetylcholine histamine release lies in the activation of muscarinic receptors, which would, in turn, inbalance the nucleotide system responsible for exocytosis. Sullivan *et al.* (1975*b*) have observed that the decrease in cyclic AMP content of rat mast cells induced by carbamylcholine is inhibited by atropine and have suggested that a muscarinic receptor is involved in this phenomenon. Our experiments using cholinergic agonists and antagonists demonstrate that the order of potency in evoking histamine release is oxotremorine > acetylcholine > carbamylcholine > nicotine, and that a competitive antagonism is observed when mast cells are challenged with acetylcholine in the presence of atropine These results indicate the presence on the cell membrane of rat mast cells of muscarinic receptors which are capable of evoking histamine release once activated by acetylcholine. A quantitative analysis of the dose–response curves shows that oxotremorine and acetylcholine have a very high affinity for the mast cell cholinergic receptors: the pD_2 value for oxotremorine is 12·72 and that for

acetylcholine 11·50. Both values have been calculated by means of the graphical method described by Van Rossum (1963) and have been found to be higher than those obtained for the pharmacological actions of these agonists in other biological systems (Van Rossum & Ariëns, 1959; Brimblecombe, 1974). The pA_2 value for atropine, on the other hand, is similar to those reported for peripheral muscarinic receptors in various tissue preparations.

TABLE 6. Comparison of the pA_2 values for atropine in different tissue preparations

Tissue	Agonist	pA_2	References
Guinea-pig lung	Acetylcholine	8·76	Arunlakshana & Schild (1959)
Guinea-pig ileum	Acetylcholine	8·4	Brimblecombe (1974)
Guinea-pig jejunum	Acetylcholine	8·1	Van Rossum & Ariëns (1959)
Rat jejeuum	Acetylcholine	8·8	Ellenbroeck, Nivard, Van Rossum & Ariëns (1965)
Rat mast cells	Acetylcholine	8.28	This paper
Frog rectus abdominis muscle	Acetylcholine	8·8	Ariëns, Simonis & Van Rossum (1964)

The ability of acetylcholine to influence biological processes in isolated cells through the activation of specific receptors has been previously demonstrated by Ignarro (1974). Acetylcholine stimulates in a dose–dependent way the phagocytic release of a lysosomal neutral protease from isolated human neutrophils. The inhibitory action of atropine suggests that muscarinic receptors are present in neutrophil cell membrane (Ignarro, 1974).

The activation of muscarinic receptors by acetylcholine and cholinergic agents has been shown to produce a significant increase in cyclic GMP levels in rat heart and human umbilical artery (George, Polson, O'Toole & Goldberg, 1970; George, Wilkerson & Kadowitz, 1973; Lyman, Sandler, Manganiello & Vaughan, 1975; Gardner & Allen, 1976). The observed increases in cyclic GMP levels were found to parallel some of the pharmacological actions of acetylcholine, such as the negative inotropic effect, and the contraction of human umbilical artery. It has also been shown that cholinergic agents, which enhance antigen-induced histamine release in sensitized human lung (Kaliner *et al.* 1972*a*; Kaliner, Orange, La Raia & Austen, 1972*b*) exert their action through an activation of guanyl cyclase, leading to the accumulation of cyclic GMP. From this evidence, it is possible to infer that also in rat mast cells acetylcholine would react primarily with a muscarinic receptor which in turn would activate, via guanyl cyclase, the synthesis of cyclic GMP, imbalancing the bidirectional system of cyclic nucleotides in favour of histamine release. Under these conditions, the increased intracellular levels of cyclic AMP obtained by treating the cells either with dibutyryl cyclic AMP or with adrenaline could restore equilibrium to the nucleotide system, in this way blocking cholinergic histamine release. Our results, showing that preincubation with dibutryl cyclic AMP and with adrenaline inhibited cholinergic histamine release, are consistent with this hypothesis and with experimental evidence obtained in rat mast cells exposed to other secretagogues (Loeffler, Lovenberg & Sjodersma, 1971; Foreman, Mongar, Gomperts & Garland, 1974; Hayashi *et al.* 1976).

The fact that carbamylcholine lowers mast cell cyclic AMP and potentiates 48/80

induced histamine release acting through a muscarinic receptor (Sullivan *et al.* 1975*a*) again points to a cholinergic mechanism modulating the spontaneous secretion of histamine.

Our results do not agree with those obtained by Kiernan (1972) and by Kazimierczak & Diamant (1978), who failed to find any secretory effect of acetylcholine on rat mast cells. The reasons for this discrepancy are not clear. There is a chance that this is due to contamination of mast cell preparation with other cells containing enzymes capable of destroying acetylcholine. In fact, when we tested the secretory effect of acetylcholine on non-purified rat mast cells, a significant degree of histamine release was obtained only if cells were exposed to acetylcholine in the presence of eserine sulphate (10^{-8} M). We studied a concentration range of acetylcholine up to 10^{-7} M and obtained a dose–response curve with a maximum effect beginning from 10^{-10} M (P. Blandina, R. Fantozzi, E. Masini & P. F. Mannaioni, unpublished).

Alternatively, a different composition of the incubation medium might contribute to explain the discrepancy. Our results have shown the critical role played by the concentrations of extracellular cations and by extracellular glucose in modulating cholinergic nistamine release.

Again, we cannot rule out that the mast cell responsiveness to acetylcholine may vary among rats of different strains and even within the same strain of rats. Using a rat strain (Sprague Dawley) different from ours, Schmutzler, Problete-Freundt, Rauch & Schoenfeld (1978) have shown that acetylcholine (10^{-12} to 10^{-6} M) induces a dose–dependent histamine release only in mast cells from actively sensitized rats. The secretory effect is blocked by atropine. Similar results have been obtained by the authors in isolated guinea pig mast cells. These observations indicate that sensitization may play a role in regulating mast cell responsiveness to cholinergic stimuli. Schmutzler *et al.* (1978) and Schmutzler (1978) have demonstrated that acetylcholine (10^{-10} to 10^{-6} M) evokes histamine release also from isolated and untreated human adenoidal mast cells. The process is dose–dependent and reaches the maximum at an acetylcholine concentration of 10^{-8} M. The maximum of this secretory response is comparable with the maximum effect we have obtained in normal isolated and purified rat mast cells. Finally, also hormonal influences may contribute to the variations in mast cell responsiveness to acetylcholine, as shown by the inhibitory action of adrenaline and cholinergic histamine release.

In conclusion, cholinergic histamine release from rat mast cells depends on the integrity of cell metabolism, the concentration of intra- and extracellular Ca and the extracellular concentrations of Na and H ions. Experimental evidence indicates that this secretory process is triggered by a muscarinic receptor acting through the alteration of intracellular balance of cyclic nucleotides. Thus acetylcholine can be considered among the variety of agents causing a selective, non-cytotoxic, histamine release from isolated purified rat mast cells.

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